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REMARKS/ARGUMENTS

The specification has been amended to replace an embedded hyperlink with an appropriate literature reference.

Claims 1-15 remain in this application. Claims 16-43 have been cancelled. Claims 1, 5, 7, 9, 10 and 12 are currently amended. Claims 18-43 were withdrawn from consideration as being drawn to a non-elected invention and are cancelled in this paper.

Concerning objections to the specification

The Examiner has objected to the specification for the use of trademarks, but has not specifically identified which terms constitute trademarks that should be identified as such. The specification indeed refers to a number of trademarked adjuvants, such as Ribi™, ImjectAlum™, and TiterMax™. But in each instance, these terms are identified as trademarks with the "TM" symbol. If there are other terms of concern to the Examiner, Applicants respectfully request that the Examiner further particularize this objection.

The hyperlink to which the Examiner has objected has been deleted.

Claims 5, 6, and 14 has been objected to as containing trademarked items. In claim 5, TiterMax™ has now been identified as a trademark. In claim 6, alum is believed not to be a trademark. In claim 14, Phospholipon 90G™ has been identified as a trademark.

Concerning 35 U.S.C § 112, second paragraph

Claims 7-11, 16 and 17 of record stand rejected under 35 U.S.C 112, second paragraph, as being indefinite. In particular, the Examiner has objected to the language "capable of" in claims 9-11, "suitable" in claims 7-11 and "long-term" in claims 16 and 17.

Claims 16 and 17 has been cancelled, rendering this rejection moot with respect to these claims.

Claim 7 has been amended to remove the term "suitable". Similarly, in claim 1, a "suitable adjuvant" has been replaced with simply "adjuvant".

The expression "is capable of eliciting" has been replaced with "elicits" in claim 9.

These amendments, made merely for the sake of clarity, are believed to fully address the Examiner's concerns.

Concerning 35 U.S.C. § 102

Claims 1-5, 7-11, 13 and 15 of record stand rejected under 35 U.S.C 102 (b) as being anticipated by Nash et al (1985) J. Reprod. Immunol., 7:151-162.

Claims 1-10, 13 and 14 stand rejected under 35 U.S.C. 102(e) as being anticipated by U.S. Patent No. 6,110,492 to Alving et al.

Claims 1-4, 7-10 and 12-17 stand rejected under 35 U.S.C 102(b) as being anticipated by Brown et al (1997) J. Reprod. Immunol. 35:53-64.

Claims 1-9 and 12-17 stand rejected under 35 U.S.C. 102(a) as being anticipated by WO 00/37100 (Brown et al).

Claims 1-5 and 7-17 of records stand rejected under 35 U.S.C. 102(b) as being anticipated by U.S. Patent No. 5,736,141 to Brown et al.

Applicants traverse these rejections and submits that the claims, as presently amended, patentably distinguish from the cited references.

Claim 1 is the sole independent claim now pending. As presently amended, claim 1 is directed to:

> A vaccine composition, comprising:
>
> (a) a carrier comprising a continuous phase of a hydrophobic substance;
>
> (b) liposomes;
>
> (c) an antigen encapsulated in said liposomes, said antigen being an antigen which, when not in said vaccine composition, has a conformation other than its native conformation, with the proviso that said antigen is other than a zona pellucida-derived antigen; and,
>
> (d) an adjuvant.

A number of amendments have been made to claim 1.

First, claim 1 has been amended to recite a "vaccine composition" rather than a "composition for use as a vaccine". This clarifying amendment addresses the Examiner's objection to the intended use recited in claim 1 of record.

Second, claim 1 has been amended to specify that the antigen is encapsulated in liposomes. Support for this amendment is found throughout the specification and claims of records, which make clear that the antigen is encapsulated in liposomes. Of course, the skilled person will appreciate that it is not necessary that every antigen particle becomes encapsulated in liposomes nor do the claims exclude the possibility of additional antigens outside the liposomes. However, as discussed herein, it is believed that the encapsulation of the antigens in liposomes contributes to the surprising advantages of the invention.

Third, as discussed in detail below, claim 1 has been amended to specify that the antigen is "an antigen which, when not in said vaccine composition, has a conformation other than its native conformation, with the proviso that said antigen is other than a zona pellucida-derived antigen."

Brown et al (1997); International Publication No. WO 00/37100; and U.S. Patent No. 5,736,141

WO 00/37100 is not prior art

WO 00/37100, cited under 35 USC 102(a) was published on June 29, 2000. Submitted herewith is a Declaration pursuant to 37 CFR §1.131, executed by the three inventors, establishing that they invented the instantly claimed subject matter prior to June 29, 2000. Accordingly, WO 00/37100 is not prior art.

Brown *et al.* (1997) and U.S. Patent No. 5,736,141 concern only zona pellucida

Brown *et al.* (1997) and U.S. Patent No. 5,736,141 (hereinafter collectively described as "the Brown references") concern Applicants' previous work in the area of immunocontraception. These references concern the use of zona pellucida (ZP) glycoproteins from mammalian oocytes for immunocontraception.

In Applicants' previous work, disclosed in the Brown references, a vaccine for the immunocontraception of mammals is described. This vaccine comprises a zona pellucida antigen and an adjuvant encapsulated in a liposome delivery system. Applicants found that the liposome delivery system allowed for the slow release of antigen, resulting in a prolonged immune response. In particular, after a single injection of the vaccine, levels of anti-zona pellucida antibodies were detected for up to 22 months in seals. Thus, this vaccine was found to be effective after a single dose and therefore very useful in immunocontraceptive protocols.

While Applicants considered that a liposome delivery system provided for slow release of the ZP antigen to provide long term immunocontraception, it was not contemplated or appreciated in the Brown references that liposomes would be useful for delivering antigens other than ZP. Indeed, as discussed below, the art in fact taught away from the use of liposomes.

More importantly, in Applicants' previous work, Applicants were not investigating the effect of the combination of liposomes and a carrier having a continuous hydrophobic phase on the conformation of the antigen. In the Brown references, Applicants were only seeking to effect long-term immunocontraception using ZP-derived antigens. The Brown references do not teach or suggest that the combination of liposomes and a carrier having a continuous hydrophobic phase would confer advantageous properties on the antigen in the vaccine, or that this advantageous combination was of general application.

It is only in the instant application that Applicants discovered that this combination maintains the antigen in a native-like three-dimensional conformation such that the instantly claimed combination of (a) a carrier comprising a continuous phase of a hydrophobic substance, (b)

liposomes, (c) an antigen and (d) an adjuvant, would be useful or advantageous with antigens other than ZP-derived antigens.

The benefit of liposome delivery of antigens was not predictable at the time of the invention

As mentioned above, the available literature at the time of the instant invention suggested that liposome delivery of antigens, other than ZP-derived antigens, would not increase production of antibodies.

For example, Powers (1997) and Powers et al (1995) report that: " the liposome vaccine was well-tolerated but elicited serologic responses that were no different in frequency or magnitude from those induced by the control vaccine." (Summary of a clinical trial with liposome-adjuvanted influenza A virus vaccine in elderly adults. Mech. Ageing Dev. 93: 179-188).

In previously immunized elderly adults, inactivated influenza A (H1N1) virus vaccines induced poor antibody responses that were not enhanced by liposomes. Vaccine 13:1330-1335 (1995).

In a study with yet another antigen, Nash et al (1995) (i.e. the cited reference mentioned above) demonstrated that administration of the beta-sub unit of human chorionic gonadotrophin linked to tetanus toxoid and absorbed onto aluminum hydroxide precipitate and delivered using liposomes resulted in only intermediate levels of antibody production.

Other studies with other antigens have demonstrated that liposome delivery of antigens does not necessarily result in increased antibody production. A survey of the literature suggests that increased antibody production is not a general property of liposome delivery of antigen.

Only in the instant application is the combination of liposomes and a carrier having a continuous hydrophobic phase recognized as being advantageous

It is only in the instant application, that the combination of a carrier comprising a continuous phase of hydrophobic substance, liposomes and adjuvant is recognized as being useful and advantageous for antigens other than ZP glycoproteins. In the instant application, Applicants have found that in the vaccine composition of the invention, antigens other than ZP produce enhanced levels of antibodies that binds to native epitopes of the target proteins. This has proven to be the case even though the antigen is a non-native, recombinant or denatured protein, or fragment thereof. As discussed in the specification at e.g. page 9, lines 18-25, Applicants believe that this may be due to the antigen being held in a native-like three-dimensional conformation in the vaccine composition.

The instant application contains extensive data concerning antigens other than ZP

In Applicants' previous work with ZP it was not recognized or disclosed that the superior results obtained by encapsulating ZP in liposomes was related to or possibly related to restoration of the native conformation of the ZP by liposomes. The instant application contains numerous examples with other antigens showing how the liposomes are believed to restore native

conformation to the antigens, whereby they elicit the production of antibodies having greater affinity for native antigens, thus making the vaccines of the invention more effective:

Alcohol dehydrogenase

Example 2 of the instant application beginning at page 21, illustrates the immunization of rabbits against native and denatured yeast alcohol dehydrogenase (ADH). ADH is an enzyme whose enzymatic activity is destroyed by heating (e.g. 100°C for thirty minutes). Heating denatures ADH by causing conformational changes. Therefore, denaturing can be measured by loss of enzymatic activity.

Example 2 shows first that ADH is a better antigen than denatured ADH because, across the board, native ADH raised higher titers of anti-ADH antibodies than did denatured ADH.

When vaccine compositions of the instant invention were used, with native ADH as the antigen, similar quantities of antibodies were raised as when a typical prior art vaccine composition using native ADH was used. That is, the advantageous effect of the instant invention on the conformation of the antigen was not invoked because the antigen was already in native conformation.

But when denatured ADH was used, vaccine compositions of the invention gave 2.7 times more antibody that bound to a native ADH than when a prior art vaccine composition using denatured ADH was used, demonstrating that the vaccine compositions of the invention better elicit antibodies having affinity to native epitopes than when antigens in non-native conformations are used.

Streptokinase

Example 8, commencing at page 40 of the specification, concerns immunization against streptokinase. Streptokinase is an antigen having immunological determinants that are resistant to change by heat treatment — i.e. they remain in their native conformation. Consequently, one would not expect to find a difference in binding of anti-streptokinase antibodies to native and heat-treated streptokinase.

These were indeed the observations Applicants noted in Table 8 on page 42 of the specification. There was no difference in the immune response of rabbits immunized with native versus heat-treated streptokinase regardless of the delivery system employed (i.e. a vaccine composition according to the instant invention or a conventional prior art vaccine).

Hepatitis B surface antigen

Example 10 in the specification, beginning at page 44, concerns immunization against hepatitis B. Hepatitis B surface antigen is produced in bacteria by recombinant means. It is well known that recombinantly produced proteins foreign to bacteria are not folded correctly, and therefore have altered conformation.

In example 10, a hepatitis B vaccine comprising a recombinant hepatitis B surface antigen was made in accordance with the vaccine compositions of the invention and compared to a corresponding conventional hepatitis B surface antigen vaccine.

As shown in Table 10, the vaccine prepared in accordance with the invention produces about six times more antibody one-month after immunization, than conventional delivery of recombinant hepatitis B surface antigen.

It should be noted that the antibody titers in this experiment were measured using native hepatitis B surface antigen, isolated at great cost from patients infected with hepatitis B. These data therefore clearly demonstrate that the antibodies produced by the vaccine compositions of the invention have greater affinity for native hepatitis antigen then do the conventional recombinant vaccines.

Epitope mapping

Example 14 commencing at page 51 of the instant specification describe epitope mapping experiments that demonstrated that vaccines of the present invention produced antibodies having different binding specificity for an antigen than achieved by conventional immunization protocols.

These experiments involved ZP, as in Applicants' previous work. Mammalian ZP is insoluble in aqueous buffers when present as the external covering of oocytes, but is rendered soluble in aqueous buffers following heating at 70-80°C for thirty minutes. The heat extracted soluble form of ZP is termed soluble intact zona pellucida (SIZP). Heating changes the structural organization of ZP, such that SIZP is soluble is aqueous buffers but less similar to ZP as presented on the oocyte surface.

The epitope mapping experiments described in example 14, demonstrate that the instant invention elicits the production of antibodies with more affinity for native ZP, particularly 3-4 months or more post-immunization, than do conventional vaccines.

In example 14, affinity of antibodies raised against conventional SIZP vaccines and SIZP vaccines of the invention were tested for affinity to various SIZP fragments. These fragments do not share the three-dimensional structure of full length SIZP.

As discussed at page 52 of the specification, Applicants found that conventional SIZP vaccines raised anti-SIZP antibodies that have a high affinity for the various SIZP fragments. In contrast, vaccines of the invention raised anti-SIZP antibodies that have low affinity for the SIZP fragments. As discussed at page 53, the antibodies raised against vaccines of the invention that bind to intact SIZP but not to fragments thereof, must either be recognizing three-dimensional structures found only on full-length SIZP protein, or carbohydrates covalently linked to these proteins. Unlike the full-length SIZP, the fragments (ZPB1, ZPB2, ZPC1, and ZPC2) are not glycosylated. As discussed at page 53, since the total amount of antibody bound to the fragments

exceeded or was equivalent to the amount of antibody binding to the intact protein, carbohydrate-recognition must have a minor role in the antibody affinity. This implies that 3-D structure determines the difference in binding to the fragments as opposed to the intact SIZP.

In another experiment discussed in example 14 (page 53, lines 19-29), 60 to 80% of anti-SIZP antibodies produced by rabbits immunized with a vaccine of the current invention, bound only to epitopes found in full-length native ZPB and ZPC. Similar results were observed in harp seals (paragraph bridging pages 53 to 54).

To summarize Example 14, conventional vaccines and vaccines according to the invention were prepared using SIZP as the antigen (i.e. zona pellucida in a denatured and non-native conformation). The affinity of antibodies raised against these vaccines for full-length intact ZP having its native conformation versus ZP fragments having non-native conformation was tested. The antibodies raised against vaccines of the invention had affinity for native ZP and not for its non-native fragments. The antibodies raised against the conventional vaccines had high affinity to denatured ZP fragments and low affinity to intact native ZP. This confirms that the vaccines of the invention restore SIZP (i.e. heat denatured in non-native conformation) to its native conformation, such that antibodies recognizing the native conformation of ZP are raised. This did not occur with the conventional vaccine.

Applicants' earlier work, as disclosed in the Brown references, did not study nor appreciate the effect of vaccine compositions of the invention on 3-D structure of the antigen, unlike instant Example 14.

Bordetella pertussis

Subsequently, Applicants have done further work, corroborating the results and conclusions reached in the instant application.

Bordetella pertussis, the causative agent of whooping cough in humans, secretes a toxin that induces macrophage apoptosis (Bachelet et al (2002) FEMS Immunol. Med. Microbiol. 32:125-131). Therefore, it would be expected that immunization against pertussis toxoid (i.e. denatured, inactivated pertussis toxin) would be possible using conventional vaccine delivery, but not using vaccine delivery in accordance with the invention. When formulated in a vaccine in accordance with the invention, one would expect that the inactivated pertussis toxoid would regain the native conformation of pertussis toxin and become active, such that it would cause apoptosis of antigen presenting macrophages, resulting in a diminished immune response. As discussed in the attached Declaration under 37 CFR §1.132 of inventor Robert Brown, these indeed were the results observed.

Presently amended claim 1 patentably distinguishes from the Brown references

In view of the foregoing, it is apparent that the instant application provides extensive evidence that the vaccine compositions of the invention restore the ability of antigens, which are not in their native conformation, to raise antibodies that recognize native antigens. This of course is a

very desirable property in a vaccine, which must protect a subject against an infectious agent that presents the antigen in its native conformation.

In Applicants' previous work involving zona pellucida, as represented by the cited Brown references, the ability of the vaccine composition to restore a non-native antigen to its native conformation was not contemplated or disclosed. Hence, it was not predictable from Applicants' previous work that the vaccine formulations of the instant invention are useful for other antigens in non-native conformation.

Claim 1 has accordingly been amended first to clarify that the antigen is encapsulated in the liposomes, and further to specify that the antigen is an antigen which, when not in the vaccine composition, has a conformation other than its native conformation, with the proviso that said antigen is other than a zona pellucida-derived antigen.

The limitation that the antigen has a conformation other than its native conformation when it is not in the vaccine composition is clearly supported throughout the specification and examples as discussed above.

The proviso excluding zona pellucida-derived antigens does not constitute new matter. The genus comprising antigens in non-native conformation is fully supported in the specification as discussed above and the specification describes numerous species within this genus. The species now excluded, i.e. ZP-derived antigens, are also discussed in detail in the specification. Thus, Applicants have taught those skilled in the art how to make a genus minus the excluded species and therefore satisfy the requirements of 35 U.S.C § 112, first paragraph. See e.g. *In re Johnson and Parnham*, 194, USPQ 187 at 196 (CCPA 1977) and also MPEP 2173.05(i).

Hence, Applicants respectfully submit that the claims, as presently amended, are fully supported by the specification as filed and clearly distinguish from Applicants' previous work as represented by the cited Brown references.

U.S. Patent No. 6,110,492 to Alving *et al.*

Claim 1 specifies, in element (a), "a carrier comprising a continuous phase of a hydrophobic substance". As discussed at pages 1-5 of the instant specification, typical vaccine compositions comprise a carrier that is an aqueous medium or an oil-in-water emulsion. In such vaccines, the antigen is necessarily suspended in the continuous aqueous phase and/or in the discontinuous hydrophobic phase.

There are significant disadvantages to the use of such carriers. When the vaccine is introduced into a subject to be immunized (an aqueous environment) antigen particles suspended in the aqueous phase are rapidly dispersed and the discontinuous hydrophilic droplets containing antigens are also rapidly dispersed in the body of the subject. Rapid dispersal of the antigen in the body of a subject through use of an aqueous or oil-in-water carrier prevents the occurrence of the desirable depot "effect". The depot effect is an improved immune response resulting from migration of antigen presenting cells to the area of the subject's body to which the vaccine has

been administered.

The instant invention achieves the depot effect through use of a carrier comprising a continuous phase of a hydrophobic substance. This continuous phase of a hydrophobic substance is immiscible with the aqueous environment of the vaccinated subject. Therefore, the carrier, and the antigens contained therein, remained localized at the site of vaccine injection, and are dispersed quickly through the body, thereby achieving the depot effect.

As discussed in detail above, the instant claims also recite the presence of liposomes, and have been amended to clarify that the antigen is encapsulated in the liposomes. Liposomes are closed lipid bilayer membranes containing an entrapped aqueous volume. As discussed above in great detail, Applicants have found that a surprisingly improved immune response is obtained when the antigen is encapsulated in liposomes and the liposomes are in a carrier comprising a continuous phase of a hydrophobic substance.

In contrast to the instantly claimed invention, Alving *et al.* do not teach a carrier comprising a continuous phase of a hydrophobic substance. Rather, as described in the abstract of the Alving *et al.* patent, Alving *et al.* describe a composition comprising a stable oil-in-water emulsion having a continuous water phase and a discontinuous oil phase. There is no teaching or suggestion in Alving *et al.* to use a carrier comprising a continuous phase of hydrophobic substance, as instantly claimed.

Alving *et al.* indicates that emulsion stability is dependent on the presence of disintegrated forms of smectic mesophase vesicles and consequently depends on unstable liposomes maintaining emulsion stability. In contrast, in the instant application, Applicants are able to obtain stable compositions comprising liposomes in a water-in-oil emulsion having a continuous oil phase and a discontinuous water phase. This is achieved e.g. through the use of a preponderance of phospholipids in the liposomes described in the Examples in the instant application. The phospholipids impart stability to the liposomes in the hydrophobic environment of the carrier, and the liposomes remain intact.

Alving *et al.* do not teach or suggest how to prepare a stable composition of liposomes in a carrier comprising a continuous phase of a hydrophobic substance and therefore do not teach or suggest the instantly claimed invention.

Nash *et al.* (1985)

Nash *et al.* demonstrate that administration of the beta-sub unit of human chorionic gonadotrophin linked to tetanus toxoid and absorbed onto aluminum hydroxide precipitate results in higher antibody production where formulated in a water-in-oil emulsion.

But only one formulation investigated by Nash et al used liposomes as the vaccine delivery vehicle (see Table 4 of Nash *et al.*), as instantly claimed. In the one experiment in which liposomes were used by Nash *et al.*, the liposomes were placed in a oil-in water emulsion (i.e. the

hydrophobic phase was not continuous) and gave only a intermediate response (see top of page 159 of Nash et al).

Thus, like Alving *et al.*, Nash *et al.* fail to teach or suggest the combination of liposomes and a carrier comprising a continuous phase of a hydrophobic substance, as instantly claimed.

Reconsideration and withdrawal of the rejections under 35 USC 102 are therefore requested.

In view of all of the foregoing, Applicants respectfully request that a timely notice of allowance be issued in this case.

Respectfully submitted,

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Date: November 21, 2003

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